

## Comparison of the effects of peripheral blood-derived and bone marrow-derived platelet-rich fibrin for osteochondral defects in rabbits

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**INTRODUCTION:** Platelet-rich fibrin (PRF) is an autologous fibrin-rich gel prepared by centrifugation of whole blood using a glass tube without anticoagulants. PRF releases several growth factors gradually and acts as a scaffold for cell proliferation. A previous study has shown that peripheral blood-derived PRF (P-PRF) promotes osteochondral repair<sup>1</sup>. Bone marrow aspirate concentrate (BMAC) contains mesenchymal stem cells and a variety of growth factors. Although BMAC has also been reported to repair osteochondral defects<sup>2</sup>, it is liquid and diffuse; it is ideal to use BMAC in combination with scaffolds for the treatment of osteochondral defects. Recently, we have reported that bone marrow-derived PRF (BM-PRF) can be made<sup>3</sup>. The advantage of BM-PRF is that it is autologous, easy to prepare, and facilitates its clinical application<sup>3</sup>. However, the therapeutic potential of the BM-PRF for osteochondral defects is unknown. We hypothesized that BM-PRF would provide better osteochondral repair than P-PRF. The purpose of this study was to compare the efficacy of P-PRF and BM-PRF for osteochondral defects in rabbits.

**METHODS:** All animal experiments were approved (protocol number: R3154) and performed following our institution's Animal Care and Use Committee guidelines. Thirty-six New Zealand White rabbits (weight: 2.5-3.0 kg, age: 16 weeks) were used. The animals were divided into three groups: the defect, P-PRF, and BM-PRF groups (n = 12 in each). In the defect group, a cylindrical osteochondral defect (4 mm in diameter and 3 mm in depth) was created on the patellar groove in the right knee using a drill. In the P-PRF group, 3 ml of blood was drawn collected from the central ear artery, transferred into a glass tube without anticoagulants, and centrifuged at 1,600 g for 10 minutes at room temperature to produce P-PRF (Fig 1A, B). P-PRF was transplanted into osteochondral defects (Fig 1C). In the BM-PRF group, 3 ml of bone marrow was aspirated from the iliac crest using an 18-gauge needle, transferred to a glass tube without anticoagulants, and centrifuged at 1,600 g for 10 minutes at room temperature to produce BM-PRF (Fig 1B, D). BM-PRF was transplanted into osteochondral defects. The knee joint was harvested at 4 and 12 weeks postoperatively to perform micro-CT and histological analyses.

**Micro-CT analysis:** The specimens at 12 weeks were scanned. The area of osteochondral defect was equally divided into 9 sagittal images, and the repaired subchondral bone area was trimmed and measured its volume using ImageJ. The subchondral bone volume was summed in a total of 9 images.

**Histological analysis:** The specimens at 4 and 12 weeks were used. After fixation and decalcification, paraffin sections were made and stained with Safranin O. The osteochondral defect was evaluated using the histological scoring system of Niederauer et al<sup>4</sup>. In addition, at 4 weeks immunohistochemical staining for TGF-β1 was performed, and the percentage of TGF-β1 positive stained area was assessed using ImageJ. In the infrapatellar fat pad synovium, paraffin sections were made, stained with hematoxylin & eosin, and evaluated using Krenn score<sup>5</sup>.

**Statistical analysis:** The scores were determined by three blinded, independent observers. The three groups were compared using a one-way ANOVA with the post-hoc Tukey test for parametric analysis and the Kruskal-Wallis test with the post-hoc Steel-Dwass test for non-parametric analysis. Analyses were performed using EZR, and  $p < 0.05$  was considered a significant difference.

**RESULTS:** Micro-CT showed that the subchondral bone volume in the PRF group at 12 weeks was greater than the other groups ( $p < 0.05$ ) (Fig.2). Histologically, Niederauer score was no significant difference among the three groups at 4 weeks. At 12 weeks, the BM-PRF group mostly healed with hyaline cartilage, whereas the P-PRF group was healed with mixed hyaline cartilage and fibrocartilage. The control group was dominantly healed with fibrocartilage. The Niederauer score in the BM-PRF group was significantly higher than the defect group ( $p < 0.05$ ), but it was no significant difference compared to the P-PRF group (Fig.3A). In the percentage of TGF-β1 positive stained area at 4 weeks, the BM-PRF group was significantly higher than the control and P-PRF groups ( $p < 0.05$ ) (Fig. 3B). The Krenn score in the BM-PRF group was significantly lower than the control group and tended to be lower than the P-PRF group at 4 weeks (Fig. 3C). The Krenn score was no significant difference among the three groups at 12 weeks.

**DISCUSSION:** This study was the first to compare the therapeutic potential of P-PRF and BM-PRF for osteochondral defects in rabbits. The transplantation of BM-PRF into osteochondral defects led to be healed with hyaline-like cartilage, which was better osteochondral repair compared to the transplantation of P-PRF and defect only. In addition, BM-PRF decreased synovial inflammation and increased the expression level of TGF-β1 in the early stage. These findings indicated that BM-PRF had an anti-inflammatory effect and accelerate the healing process of osteochondral defects.

**SIGNIFICANCE/CLINICAL RELEVANCE:** BM-PRF has the potential to improve clinical outcomes for the treatment of osteochondral defects.

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